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THE LEVELS OF ADENOSINE TRIPHOSPHATE SYNTHASE 6, MANGANESE SUPEROXIDE DISMUTASE, NADPH-CYTOCHROME P450 REDUCTASE, LACTATE DEHYDROGENASE IN SEMINAL PLASMA OF OLIGOZOOSPERMIA AND NORMOZOOSPERMIA MEN

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ABSTRACT

Objective: *In recent years, energy production and enzymes in mitochondrial pathways are an important in male fertilization.*

Material and Method: *For this purpose, we tried to determine the relationship between normozoospermia and oligozoospermia individuals according to levels of some mitochondrial enzymes. The seminal plasma levels of mitochondrial encoded adenosine triphosphate synthase 6 (mtATP6), manganese superoxide dismutase (Mn-SOD), NADPH-cytochrome P450 reductase (CPR), lactate dehydrogenase (LDH) were analyzed and assessed their correlations between data of spermogram. The study was composed of two groups consisting of normozoospermic and oligozoospermic volunteers.*

Results: *mtATP6 levels were significantly higher in normozoospermia (0.97±0.50 ng/mg protein, p=0.008) than oligozoospermia (0.64±0.32 ng/mg protein). LDH levels were higher in normozoospermia (0.25±0.074 U/mg protein, p=0.007) than oligozoospermia (0.199±0.049 ng/mg protein). The groups did not differ in terms of MnSOD and CPR levels. mtATP6 levels were significantly correlated with sperm concentration, total number and total motility, immotility, total progressive motile sperm count (TPMSC) and long head anomaly. LDH levels were related with long head and short tail anomaly.*

Conclusion: *We observed some of the spermatogenetic anomalies are caused by defects in energy regulation. We did not find any evidence on MnSOD and CPR activity regarded as determinative parameters in response to increased oxidative stress in oligozoospermia.*

Keywords: *Infertility, MnSOD, LDH, mtATP6, CPR.*

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OLİGOSPERMİLİ VE NORMOSPERMİLİ ERKEKLERİN SEMİNAL PLAZMALARINDA ADENOZİN TRİFOSFAT SENTAZ 6, MANGANEZ SÜPEROKSİD DİSMUTAZ, NADPH-SİTOKROM P450 REDÜKTAZ VE LAKTAT DEHİDROJENAZ SEVİYELERİ

ÖZET

Amaç: Son yıllarda mitokondriyal yolaklardaki enzimler ile enerji üretimi ve erkek fertilizasyonu arasında önemli bir ilişkinin olabileceği bildirilmektedir.

Materyal ve Metot: Bu amaçla, normospermili ve oligospermili bireylerde mitokondriyal bazı enzimlerin düzeylerini inceleyerek aralarındaki ilişkiyi araştırmaya çalıştık. Mitokondriyal-kodlanmış adenozin trifosfat sentaz 6 (MTATP6), manganez süperoksit dismutaz (Mn-SOD), NADPH-sitokrom P450 redüktaz (CPR), laktat dehidrojenaz (LDH)'ın seminal plazma düzeyleri analiz edildi ve spermiyogram verileri arasındaki korelasyon değerlendirildi. Çalışma normospermik (n=30) ve oligospermik (n=30) gönüllülerden oluşan iki gruptan oluşturulmuştur.

Bulgular: mtATP6 seviyeleri normozoospermililerde (0,97±0,50 ng/mg protein, p=0,008) oligozoospermililerden (0,64±0,32 ng/mg protein) ve de LDH seviyeleri normozoospermide (0,25±0,074 U/mg protein, *p*=0,007) oligozoospermiden (0.199±0.049 ng/mg protein) anlamlı düzeyde daha yüksekti Gruplar, MnSOD ve CPR düzeyleri açısından farklılık göstermemiştir. mtATP6 seviyeleri, sperm konsantrasyonu, total sayı, total motilite, immobilite, total progresif motile sperm sayısı (TPMSC) ve uzun baş anomalisi ile önemli ölçüde korelasyon göstermiştir LDH düzeyleri uzun baş ve kısa kuyruk anomalisi ile ilişkilidir.

Sonuç: Bazı spermatogenetik anomalilerin enerji düzenlemesindeki kusurlardan kaynaklandığını gözlemledik. Oligozoospermide artan oksidatif strese yanıt olarak belirleyici parametreler olarak kabul edilen MnSOD ve CPR aktivitesindeki değişimler bakımından herhangi bir bulgu tespit etmedik.

Anahtar kelimeler: İnfertilite, MnSOD, LDH, mtATP6, CPR.

INTRODUCTION

Infertility is defined as not being able to produce children despite one year of regular and unprotected sexual intercourse and is seen in approximately 15% of couples.[1] Half of the male cases are associated with inaccurate spermatogenesis identified as an oligospermia and azoospermia.[2] Oxidative stress-induced DNA damage is one possible cause that seriously affect sperm quality, and damages are seen in nuclear and mitochondrial levels.[3,4] In addition, oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system in the cells. Recent studies indicate that ROS is associated with reduced fertility due to high polyunsaturated fatty acids levels in the sperm plasma membrane, which are toxic substances in human spermatozoa.[4-6] However, small amounts of ROS are needed for spermatozoa to achieve fertilization.[7]

Sperm specific gene mutations, such as point mutation and mitochondrial DNA (mtDNA) deletions are related to low sperm motility and semen quality due to the amount of ROS produced during oxidative respiration.[4] When a large amount of mutant mtDNA accumulates in the testicles, a decrease in adenosine triphosphate (ATP) production, mitochondrial respiratory dysfunction and meiotic arrest are induced in spermatogonia cells.[8] The mitochondrial respiratory chain (RC) catalyzes the oxidation of fuel molecules and the concomitant energy transduction into ATP via five enzyme complexes of oxidative phosphorylation (OXPHOS), which are embedded in the inner mitochondrial membrane.[9,10] Mitochondrial ATP synthase subunit A (ATP synthase F0 subunit 6, mt-ATP6) plays an important role in ATP synthesis, in which mutations affect ATPase 6 and cause human diseases.[11] The a2 isoform of vacuolar-ATPase (ATP6V0A2) is required for normal spermatogenesis and maturation of sperm. This multi subunit enzyme is a proton pump that regulates the acidification of the intracellular compartment and the extracellular environment.[12] V-ATPases in the plasma membrane activates sperm motility and are responsible for acrosomal site acidification, regulates the intracellular pH of organelle.[13,14] ATP6V0A2 is a putative endosomal pH-sensor.[15] Another important function of the ATP6V0A2 protein is the regulation of testicular immune response and apoptotic pathways in spermatogenesis.[12,16] Ota *et al.* (2013) showed a relation between ATP6V0A2 expression and cytokine secretion in human semen.[17] Abnormal spermatogenesis caused by disrupted chemokine/cytokine network and apoptotic pathways causes low sperm motility and poor sperm production.[12]

Superoxide dismutase (SOD) is a metalloenzyme, an antioxidant that catalyzes radicals to hydrogen peroxide (H_2O_2, which is further detoxified by glutathione

peroxidase (GSH-Px) and catalase (CAT) with the cyclic oxidation and reduction reactions.[18] The SOD family has three forms: the cytoplasmic form is SOD_1, the mitochondrial manganese (Mn) type SOD_2 and Cu-Zn type is the extracellular SOD_3. Mn-SOD_2 is a homotetramer with each subunit containing an active site surrounding the Mn ion.[19] Superoxide dismutase 2 (Mn-SOD_2), is the main antioxidant enzyme in the mitochondrial matrix that destroys ROS and valuable enzymes analyzed in the evaluation of mitochondrial oxidation.[20,21]

NADPH-cytochrome P450 reductase (CPR) is an important redox partner for all microsomal cytochrome P450 monoxygenases, which metabolizes a large number of endogenous and exogenous compounds.[22,23] Aromatase is a cytochrome P450 enzyme responsible for converting androgens to estrogens found in the reproductive system, adipose tissue, liver, brain, and testicles. The aromatase enzyme is also found in germ cells with Leydig and Sertoli cells in the testis, and an indicator of the phenotype of undifferentiated mesenchymal cells.[24] It has a microsomal complex structure consisting of two proteins: the cytochrome P450 aromatase enzyme which contains a steroid binding pocket and heme, and the NADPH-cytochrome P450 reductase enzyme.[25,26]

LDH enzyme converts pyruvate to lactate in Sertoli cells.[27,28] Lactate is a critical "fuel" in the development of germ cells.[29] Serum levels of LDH is an important biomarker in clinical examination.[30] LDH isoenzymes occur with different kinetic properties. Prepubertal men do not have this enzyme, and it appears that there is a link between human testis specific L-lactate dehydrogenase-4 (LDHC4) and spermatogenesis. The level of LDHC4 increases with testicular maturation.[31] Spermatozoa LDHC4 uses lactate as the main energy source. It is a sperm specific enzyme located on the plasma membrane of the sperm and displays a different distribution compared to other somatic LDH isoenzymes. Antibodies developed against LDHC4 suppress fertility in at least two ways; by damaging sperm energy metabolism and by destroying sperm functions. Surface of the LDHC4 can contribute to sperm agglutination with its antibody binding sites such as complement-assisted cytotoxicity.[32] Sperm transport is significantly inhibited when anti-LDHC4 serum is transferred to the female reproductive tract.[33] Pathological disorders in spermatogenesis also cause failure in LDH-C4 activation. Velasco *et al.* showed that LDHC4 in seminal fluid may be evaluated as an infertility index for men.[34] Furthermore, LDHC4 is an important clinically biomarker in the evaluation of germinal activity and spermatozoid quality.[28]

There are limited studies investigating basic molecular mechanisms, such as redox balance, and oxidant-antioxidant mechanisms on fertility. Many researches have studied the expression of these parameters in the sperm. However, these biochemical parameters and spermogram remains unelucidated. Based on these, the present study is considered to evaluate the importance of seminal plasma mtATP6, MnSOD, CPR, and LDH ratio in infertile men. Furthermore, this study aimed to determine mtATP6, MnSOD, CPR, and LDH levels in normozoospermic and oligozoospermic individuals to clarify the mitochondrial process and overview of the redox pathway and energy production in infertility. In addition, the relation between spermogram data and mentioned biochemical parameters were evaluated.

MATERIAL AND METHOD

Individuals

The present study was performed on semen samples taken from volunteers aged 23 –51 years old who were admitted to In Vitro Fertilization (IVF) Unit Andrology Laboratory of Medicine Faculty Hospital at University, Konya, Türkiye. All the volunteers signed the "Informed Patient Consent Form" as written to the ethical standards and the Declaration of Helsinki Principles and approved by Medicine Non-Interventional Clinical Research Ethics Committee of Selcuk University. The study was conducted with permission of Medicine Non-Interventional Clinical Research Ethics Committee of Selcuk University with the number of 2019/259 (decision date: 16.10.2019).

Participants consist of men in couples which pregnancy did not occur despite regular sexual intercourse in their reproductive period for at least 1 year without using any contraceptive.

Sixty male volunteers were included in the present study and the groups were formed as follows (Table 1);

Group 1: Individuals with normozoospermia (n=30), Sperm count≥15 million/mL,
Group 2: Individuals with oligozoospermia (n=30), Sperm count<15 million/mL. The average age of individuals in group 1 was 31.17±5.02 while that of group 2 was 32.97±7.01 years (mean±SD).

The groups were evaluated according to Kruger criteria (semen volume: 1.5 mL; sperm concentration: 15 million / mL; total sperm count: 39 million; total sperm motility: 40%; progressive sperm motility: 32% A±B; and sperm morphology: 4%). The percentages of normal and abnormal spermatozoa forms were determined by scoring at least 100 spermatozoa

Table 1. Data of Spermogram used to compose grouping as normozospermia and oligozospermia male and total protein concentrations (mean±SD)

Semen paremeters	Normozoospermia (n=30)	Oligozoospermia (n=30)	*p* value
Age (years old)	31.17±5.02	32.97±7.01	0.354
Volume (mL)	3.69±1.25	4.7±1.29	0.007**
Concentration (million /mL)	50.27±23.31	11.33±2.42	0.000**
Total number (million)	188.64±96.89	51.03±14.48	<0.001
Total Motility (%)	68.91±8.63	67.33±9.25	<0.001
Progressive Motility (%)	55.41±9.82	50.33±11.96	<0.001
Non Progressive Motility (%)	13.5±4.17	17±5.73	0.019*
Immotility (%)	31.09±8.63	32.67±9.25	<0.001
TPMSC (Million)	108.62±62.19	25.83±9.93	<0.001
Normal Morpholgy (%)	2.27±0.70	1.33±0.52	0.001**
Head Anomaly (%)	88.96±1.96	91.17±1.84	0.004**
Amorphous Head (%)	76.05±4.95	80.50±4.59	0.042*
Large Head (%)	5.14±3.39	5.50±5.68	0.711
Small Head (%)	2.64±1.94	1.50±1.52	0.199
Long Head (%)	4.18±3.05	3.17±2.56	0.411
Multiple Head (%)	0.96±1.46	0.50±0.84	0.891
Neck or tail anomaly (%)	8.86±1.46	7.50±1.52	0.037*
Neck-Middle Piece Anomaly (%)	12.86±2.93	17.83±3.19	0.001**
Tail Anomaly (%)	13.68±3.40	14.17±3.06	0.565
Double Tail (%)	1.36±1.33	0.83±1.17	0.625
Tail Stump (%)	0.27±0.55	0.5±0.84	0.740
Dag Defect(%)	5.77±2.11	6.83±2.64	0.625
Long Tail (%)	0.27±0.63	0.5±0.84	0.325
Short Tail (%)	5.91±2.00	5.50±1.52	0.765
Teratozoospermia Index	1.28±0.05	1.33±0.02	0.002**
Protein (mg/ml)	5.70±1.36	5.50±1.33	0.569

TPMSC: Total progressive motile sperm count, **mean±SD:** mean standart deviation,
*: Correlation is significant at the $p<0.05$ level (1-tailed); **: Correlation is significant at the $p<0.01$ level (2-tailed)

per preparation. Spermiogram was performed at Medical Faculty Andrology Laboratory within Selcuk University.

Individuals who were under any medication (regular drug), with chronic illness (diabetes mellitus, hypertension, cardiovascular immunosuppressive, and respiratory diseases), azospermic (no sperm in the ejaculate), alcohol consumers, and cigarette smokers were excluded from the study.

Collection and storage of sperm samples and evaluation of spermogram

After a ban on coitus lasting between 2 and 6 days, the sperm sample obtained by hospital masturbation method was collected in a special sterile plastic container. Samples were started to be evaluated for liquefaction after being kept in an incubator at 37° C for 20 min. If liquefaction did not occur within 20 min., the sample was left for an additional 15 min. in the incubator. Physical properties such as sperm concentration, motility, progressive motility and immobility in sperm samples were evaluated according to the WHO (2010) criteria.[35] Smear preparations from semen samples were prepared and stained with "Spermac" stain for morphological evaluations.

Biochemical analysis

A 300 µl of the remaining semen samples were taken into falcon tubes after Spermogram analysis and centrifuged at 1,000 rpm for 20 min. The supernatant portions of the samples were separated and collected into the Eppendorf tubes. Samples of the seminal plasma were kept at –80°C until analysis. Analyses were performed according to the manufacturer's procedure. Analyses of mtATP6 (MyBiosource, cat no: MBS9325043), MnSOD activity (Abcam, cat no: ab178012), CPR (Elabscience, cat no: E-EL-H0359), and LDH activity (Elabscience, cat no: E-BC-K046) were determined by commercial test kits. Protein analyses were performed using a colorimetric commercial test kit (Thermo, cat. no: 23227) at 562 nm wavelength, and calculated as mg/mL. The results of MnSOD, mtATP6 and CPR were calculated as ng/mg protein, and LDH samples were determined as U/mg protein. Detection range of LDH test is 6-1,000 U/L. Intra-assay CV is 1.8 % and the inter-assay CV is 2.4 %. The detection range of MnSOD test is 0.22 - 50 ng/mL. Intra-assay precision CV is 3.8 % and the inter-assay precision CV is 4.2%. The detection range of the mt-ATP6 test is 0.625-20 ng/mL. Intra-assay and inter-assay CV is 15 %. Detection range of CPR test is 0.16-10 ng/mL. Intra-assay and inter-assay CV is 7 %. These parameters were analyzed at Selçuk University Biochemistry and Physiology Research Laboratories. All analysis was conducted with Elisa Reader BMG LABTECH (Germany) and Rayto Microplate washer (RT-2600, China).

Statistical Analysis

Statistical analysis was performed the using Statistical Package for the Social Sciences (SPSS 21.0) program. Shapiro-Wilk test was used to compare and check normal distribution level. independent samples t test was applied to values with $p>0.05$, and Mann Whitney U test was applied to those with $p<0.05$. While the Pearson correlation coefficient was used for $p>0.05$, the correlation coefficient of spearman was used for $p<0.05$.

Ethical issues

Ethical issues (including plagiarism, data generation and/or falsification, duplicate publication and/or submission, redundancy, etc.) have been thoroughly reviewed by all authors. Ethical and patient consent is not required. The study was carried out in accordance with the Helsinki Declaration Principles.

RESULTS

In the present study, Table 1 shows the findings of spermiogram and total protein values of the groups. The comparison of biochemical markers in semen analysis of the groups with normozoospermia and oligozospermia and their correlation coefficient values and p values with sperm parameters were evaluated in Table 2 and Figure 1-4.

Table 1. Data of Spermogram used to compose grouping as normozospermia and oligozospermia male and total protein concentrations (mean±SD).

Whereas value of mtATP6 was 0.97±0.50 ng/mg protein in seminal plasma of the normozoospermic group, it was determined as 0.64±0.32 ng/mg protein in oligospermic individuals as it shown in Figure 1. mtATP6 value in normozoospermic group was statistically higher when compared to oligozoospermic group (p=0.008).

Ratio of MnSOD in normozoospermic individuals was 8.46±2.08 ng/mg protein, the value in oligozoospermic individuals was determined as 8.15±2.03 ng/mg protein. As it shown Figure 2, there was no statistically significance difference between the groups (p>0.05).

CPR ratio was found as 2.58±0.58 ng/mg protein in normozoospermia and 2.36±0.44 in oligozoospermia group (p>0.05, Figure 3). The groups did not differ in terms of CPR values.

The value of activity of LDH enzyme was significantly higher in normozoospermic individuals (0.25±0.074 U/mg protein) than oligozoospermic (0.199±0.049 U/mg protein) individuals (p=0.007, Figure 4).

As it shown in Table 2 (n=60), when semen parameters and LDH correlations are evaluated in total group; Positive correlation at p<0.05 level between LDH and long head anomaly (r=0.342), negative correlation at p<0.01 level with short tail anomaly (r=-0.456) were determined.

When we evaluate the correlations between MnSOD and semen parameters in Table 2, the negative correlation between tail anomaly (r=-0.428, p<0.01) and short tail anomaly (r=-0.359, p<0.05) was found.

Table 2. Correlations of the semen parameters with LDH, MnSOD, CPR and MTATP6 levels in total group (n=60)

Semen paremeters	LDH	MnSOD	CPR	MTATP6
Concentration (million/ml)	ns	ns	ns	p<0.001 r=0.520
Total Number (million)	ns	ns	ns	p<0.001 r=0.468
Total Motility (%)	ns	ns	ns	0.046* r=0.286
Progressive Motility (%)	ns	ns	ns	0.017* r=0.339
Immotility (%)	ns	ns	ns	0.044* r=-0.289
TPMSC (million)	ns	ns	ns	0.001** r=0.453
Long Head (%)	0.035* r=0.342	ns	0.012* r=0.403	0.037* r=0.395
Tail Anomaly (%)	ns	0.007** r=-0.428	ns	ns
Short Tail(%)	0.004** r=-0.456	0.027* r= -0.359	0.02* r=-0.375	ns

TPMSC: Total progressive motile sperm count, **ns:** statistically none significant,
*: Correlation is significant at the p< 0.05 level (1-tailed); **: Correlation is significant at the p< 0.01 level (2-tailed)

In Table 2 shows the relations between CPR and semen parameters with long head (r=0.403) and short tail (r=-0.375) at p<0.05 level.

The correlations with mtATP6 levels and sperm concentrations, total number (million) were significantly important, respectively (r=0.520, r=0.468, p<0.01). A positive correlation with total motility (r=0.286), progressive motility (r=0.339) and mtATP6 were found (p<0.05). Immotility (%) of the sperm showed negative correlation with mtATP6 ratio (r=-0.289, p<0.05). A positive relations between total progressive motil sperm count (million) and mtATP6 levels were determined (r=0.453, p<0.01), whereas long head anomaly (%) showed a positive correlation (r=0.395, p<0.05) (Table 2).

DISCUSSION

In this study, we determined the alterations in seminal plasma of LDH enzyme activity and mitochondrial ATPase in normospermic and oligospermic men. The mtATP6 and LDH activity in the oligozoospermia group was lower than those in the normozoospermia group. MnSOD and CPR levels did not show significant differences between the groups. mtATP6 levels showed relations between sperm concentration, sperm total number and motility, and TPMSC. LDH levels were correlated with short tail and long head anomaly in both groups.

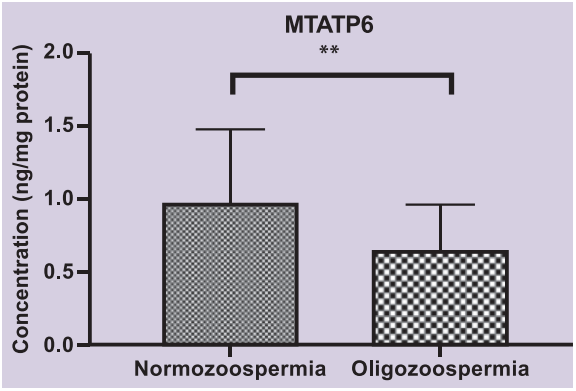

Figure 1. mtATP6 ratio (ng/mg protein) in groups (mean±SD)
mean±SD: mean standart deviation,

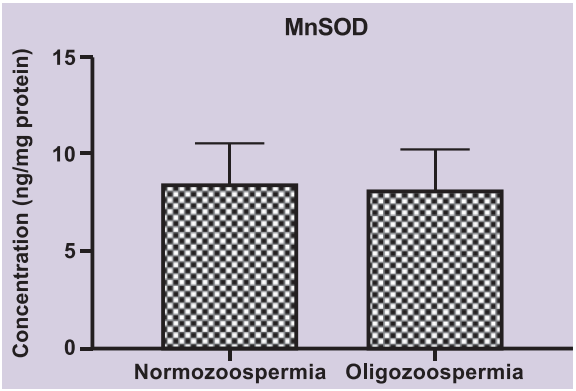

Figure 2. MnSOD ratio (ng/mg protein) in groups (mean±SD)
mean±SD: mean standart deviation,

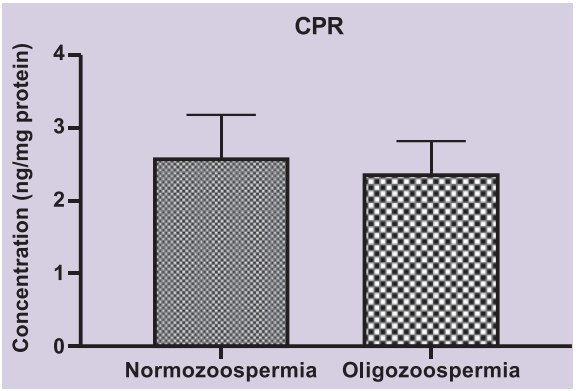

Figure 3. CPR ratio (ng/mg protein) in groups (mean± SD)
mean±SD: mean standart deviation,

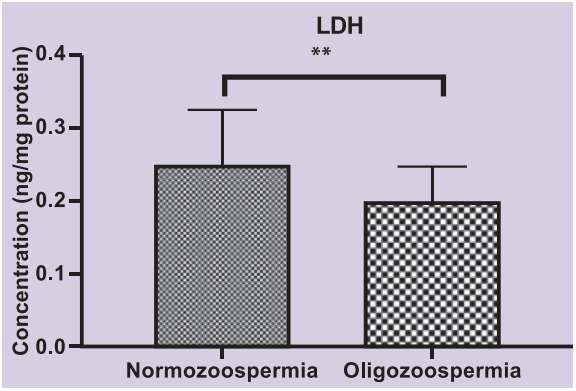

Figure 4. LDH ratio (U/mg protein) in groups (mean±SD)
LDH: lactate dehydrogenase, **mean±SD:** mean standart deviation,

Infertility is a major clinical challenge that should be solved. However, researchers focused on the physiological and pathophysiological state of the problem. Therefore, defects in sperm cell structure and molecules as the causes of male infertility and factors affecting fertilization potential, are among the subjects under investigation.

The mechanism of sperm cell metabolism and the oxidant-antioxidant system in sperm function are issues that remain unelucidated in infertility.

Mammalian spermatozoa expend energy by generated as intracellular ATP.[36] There are two pathways for ATP production in mammalian sperm, glycolysis and mitochondrial respiration. The glycolysable substrate plays an important role in sperm capacitation and zona pellucida penetration.[36-39] Mukai and Okuno supposed that -ATP is required for sperm flagellar movement, and sperm motility can be maintained in the presence of respiratory substrates in case of glycolysis.[40] In the absence of a phosphocreatine shuttle in sperm, glycolysis is activated to provide ATP along the movement of sperm tail.[36,40]

In another study conducted by Duan *Et al*, Oxamate, an inhibitor for LDH activity was used to indicate whether this enzyme serves a critical role in sperm capacitation, the acrosome reaction, and/or fertilization.[32] They determined an inhibition of LDH activity blocked capacitation. Oxamate and N-isopropyl oxamate inhibited the tyrosine phosphorylation of proteins during the sperm capacitation process as shown by western blotting. The oxidation of reduced NAD with the conversion of pyruvate to lactate by LDH provides ATP necessary for protein kinase A (PKA) activity. The inhibition of LDH3 by oxamate restricts sperm motility and capacitation.[32] Previous studies have reported that LDH levels are higher in individuals with oligospermia than those with normospermia, and are inversely proportional to concentration.[41,42] We found that LDH levels were low in patients with oligospermia and showed a positive correlation with long head anomaly and a negative correlation with short tail anomaly. Therefore, our results are consistent with the findings in previous studies. Our findings about mtATP6 also support the results of LDH. Oligozoospermic individuals have lowest LDH and mtATP6 levels and those parameters are related with one or more morphological abnormalities. Therefore, these results show that ATP is important to maintain the sperm quality. However, we did not find any evidence on the relationship between sperm tail and ATP (Table 5) as those mentioned in other studies. In addition, Ganetzky *et al.* determined that although ATP synthetic rate is constantly decreased, it is not a multipurpose

finding and cannot currently be planned as a clinical diagnostic test in patient tissues or cells. [36,41,43] The mitochondrial complex V enzymatic assay which is more established, measures the reverse reaction that is frequently preserved in tissues with pathogenic MT-ATP6 variants. MT-ATP6 variation, particularly within the linker regions between the transmembrane domains of the protein, is a common clinical quandary.[43] Feng *et al.* studied on azoospermia and normospermia groups in MTATP6 gene mutations and deletions, and found MTATP6 gene deletion with 5% and G8887A mutation in the MTATP6 gene with 20% of the azoospermia individuals (n=80).[44] Certainly, ATP cannot be evaluated as a clinical diagnostic test, although it gives information about the functioning of the metabolic process in infertile individuals. Reduced ATP levels preserve ATP hydrolysis capacity. Our results regarding spermogram relations show that ATP6 levels are correlated with sperm concentration (million/mL), the total number of sperm (million), total motility, and progressive motility. Therefore, higher ATP levels contribute to fertilization. The relationship of ATP6, especially with sperm concentration and motility, is an important finding to solve the problem of fertility.

There are different determinations on the role of SOD activity on sperm viability. Studies reported that 1-SOD prevents oxidative damage in sperm, 2-decreased SOD activity in seminal plasma is associated with male infertility, 3-decreases SOD amount that is responsible for sperm motility and vitality.[45-47] Furthermore, a high SOD ratio prevent the loss of motility in mouse sperm and the addition of SOD to sperm suspension significantly improves sperm motility.[48,49] Murawski *et al.* documented a significantly lower semen SOD activity detected in patients with oligoasthenozoospermia than those with normospermia.[50] SOD activity was higher in normospermia and had a negative correlation with the DNA fragmentation index.[51] In our study, MnSOD levels were higher in the normospermia group, although without statistical significance. In addition, high levels of SOD activity have been found in normozoospermia than in the group with a pathological spermogram.[52] SOD activity in seminal plasma and spermatozoa lysate was significantly lower in the oligospermia group than in the control group.[53] The differences between our findings and those from other studies may be due to the difference in the number of cases and in the analyses of SOD isoforms. We analyzed the mitochondrial form of SOD, whereas other researchers analyzed the total SOD. The protein expressions of CuZnSOD, MnSOD, and catalase in seminal plasma showed strong positive correlation with sperm concentration and progressive motility.[54] Our results revealed a negative correlation with tail anomaly and MnSOD activity. Macanovic *et al.* and Bykova *et al.* determined reverse results compared to our findings.[54,55] They found high SOD activity in oligospermia individuals than those with normozospermia, and activities of SOD were higher in men with low motility (<50%) than those with high motility.[55,56] Tang *et al.* reported the effects of Qiangjing Decoction on antioxidant and energy metabolism in oligospermia and astenospermia: SOD and GSH-Px levels were significantly lower, but MDA levels were significantly higher in model rats, whereas LDH level was significantly decreased in the oligoasthenospermia group compared to control group normal rats.[57]

Cytochrome P450 activates, neutralizes, and facilitates the excretion of most xenobiotics, thereby modulating the duration and intensity of their toxicity. Cytochrome P450 induces ROS that permanently impairs sperm function, thereby causing a decrease in sperm count in males and experimental animals.[58] In our results, CPR levels were higher in the normospermia group and were positively correlated with long head anomaly. Therefore, our results on the correlations of sperm anomaly show that defects in modulating the toxicity cause head anomaly but do not affect the fertility status. We did not find evidence that MnSOD and CPR activity were determinative parameters in response to increased oxidative stress in oligozoospermia.

When genomics, epigenetics, proteomics, and metabolomics are better understood, we will find the answer to the concerns about male infertility. Furthermore, it will reveal whether the biomolecular analysis used in ejaculated sperm is better than that used in testicular sperm.[59]

CONCLUSIONS

This study has limitations. We did not implement an immunohistochemical analysis. Therefore, studies involving a larger number of volunteers with sub-group defined as severe and very severe oligospermia and genomic studies are needed to clarify the defects in energy consumption. Furthermore, the biochemical analysis data may be supported by immunohistochemical methods to clarify the expression of LDH, CPR, mtATP6 and MnSOD proteins in seminal plasma.

Our study revealed that defects in energy regulation might cause spermatogenetic anomalies.

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*The authors declare that there are no conflicts of interest.

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